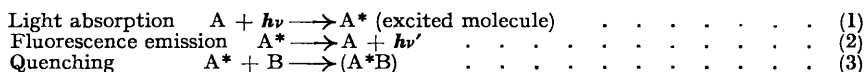


150. Some Observations on the Photochemistry of Fluorescent Substances. Part I. The Quenching of Fluorescence by Nitric Oxide and the Photochemical Formation of Nitroxides.

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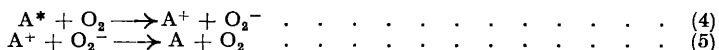
The quenching of the fluorescence of polycyclic hydrocarbons in solution by nitric oxide is described and discussed. In character and extent it is analogous to the effect of oxygen. An attempt was made to isolate stable products of the photochemical reaction between hydrocarbons and nitric oxide, but owing to the apparently great instability of the primary "photo-nitroxides" only a few milligrams of several nitrogenous products were obtained in crystalline form.

It has been suggested that the primary process of fluorescence quenching in solution by foreign substances is in many cases due to an electron transfer between the excited molecule and the quenching substance (Weiss, *Trans. Faraday Soc.*, 1938, **34**, 451; 1939, **35**, 48; Carter and Weiss, *Proc. Roy. Soc.*, 1940, *A*, **174**, 351; *Nature*, 1943, **152**, 176). Some aspects of this interpretation have also been confirmed by work on certain solid phosphors (Lewis *et al.*, *J. Amer. Chem. Soc.*, 1941, **63**, 3005; 1943, **65**, 2419). In general, the sequence of reactions can be represented by the following equations (A = fluorescent molecule, B = quenching agent) :



with a subsequent electron transfer between A^* and B resulting in either (A^+ and B^-) or (A^- and B^+). Thus the increased reactivity of the molecule in the excited as compared with the ordinary state manifests itself in one of two ways : (a) An electron transfer from A^* to B, favoured by the decrease of the ionisation potential of the excited dye, approximately corresponding to the optical excitation energy ($h\nu$); this is the mechanism in the case of polycyclic hydrocarbons, chlorophyll, tryptaflavine adsorbates, etc. (where there are non-localised π -electrons in the conjugated systems of double bonds), if the quenching agent has electron-accepting properties (*e.g.*, oxygen). (b) An electron transfer from B to A^* owing to the increased electron affinity of the excited molecule (or ion), *e.g.*, in the case of methylene-blue and other dye cations, if the quencher has electron-donating properties (*e.g.*, the ferrous ion).

The quenching of fluorescence by oxygen is represented by (4) and the regeneration of the initial state by (5) :

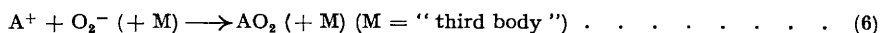


If oxygen is removed by evacuation, or replaced by an atmosphere of pure nitrogen, the reaction is reversible and the fluorescence is restored. This was first found by Kautsky for tryptaflavine gel and chlorophyll solutions (*Ber.*, 1931, **64**, 2677), and later also for aromatic hydrocarbons by Bowen and Williams (*Trans. Faraday Soc.*, 1939, **35**, 765), Weil-Malherbe and Weiss (*Nature*, 1942, **149**, 471), and Miller and Baumann (*J. Amer. Chem. Soc.*, 1943, **65**, 1540).

It was to be expected that other gases with an electron affinity would also have the ability to quench fluorescence. A number of gases were investigated by Miller and Baumann (*loc. cit.*). Sulphur dioxide proved to be a much more potent quencher than oxygen, possibly owing to its higher solubility in light petroleum. It is, however, noteworthy that polycyclic hydrocarbons form molecular compounds with sulphur dioxide in the liquid (*cf.* Weiss, *J.*, 1942, 245), so it is possible that the quenching by sulphur dioxide is not a photochemical, but a dark reaction, though there is at present some difficulty in deciding between the two mechanisms.

We investigated a number of gases and found a strong and reversible quenching effect by nitric oxide (*Nature*, 1943, **151**, 449), the extent (Table I) being very similar to that which we found with oxygen (*ibid.*, 1942, **149**, 471); it varies somewhat from one hydrocarbon to another and also with the solvent, probably owing to differences of solubility. An exception to the analogy with oxygen is observed in the case of rubrene, the fluorescence of which is inhibited to the extent of 68% and 5% by oxygen and nitric oxide, respectively. The reversibility is incomplete in the case of 20-methylcholanthrene, but is almost quantitative with the other compounds investigated.

Although the fluorescence quenching by oxygen and other gases is completely reversible, it is well known that anthracene derivatives, on prolonged irradiation, form stable peroxides of the type AO_2 (*cf.* Dufraisse, *Bull. Soc. chim.*, 1939, **6**, 422; Cook and Martin, *J.*, 1940, 1125), thus supporting the proposed mechanism of the quenching reaction. The stabilisation of the primary bimolecular association product is in general brought about by a "third body" according to :



(see Weiss, *Trans. Faraday Soc.*, 1942, **38**, 366). In solution, every collision between two solutes is practically a ternary collision, but probably some molecules are more efficient for the stabilisation of the reaction product than others.

The yield also depends on the extent of secondary photochemical decomposition of the peroxide. The particularly good yields of photo-oxides, when carbon disulphide is used as the solvent, may be explained by

the strong absorption of the wave-lengths below 3800 Å. by this solvent, which thus acts as a light filter for the short wave-lengths which would otherwise be absorbed by the photo-oxides.

In our attempts to isolate a stable product of the photochemical reaction between polycyclic hydrocarbons and nitric oxide, we first used carbon disulphide as solvent. The results were, however, no more favourable than when the solvent was hexane, and this was used in all subsequent experiments. The hydrocarbons chosen were anthracene and 9 : 10-dimethyl-1 : 2-benzanthracene, as it is known that *meso*-substituted anthracene derivatives are especially reactive in addition reactions of the kind investigated. Under the conditions employed, about 80% of hydrocarbon was recovered unchanged after an irradiation of 4—5 hours in an atmosphere of nitric oxide. The remainder was a mixture which by chromatographic adsorption analysis was resolved into approximately 30 components. The distribution of the various fractions and the general character of the chromatogram were very similar with both hydrocarbons. The bulk of the reaction products was made up of nitrogen-free compounds. In the anthracene experiment a considerable amount of anthraquinone was isolated. Other nitrogen-free compounds, from their high adsorption affinities, high melting points, and insolubility, appeared to be polymerisation products. One crystalline fraction which was isolated from the deposit that had formed from anthracene on the walls of the vessel during irradiation, was judged by its appearance, slight solubility, and melting point to be dianthracene. As we were mainly interested in the nitrogenous compounds, the nitrogen-free substances were not further investigated. It is probable, however, that they originated, at least in part, from "photo-nitroxides" by secondary decomposition, for irradiation under similar conditions in an atmosphere of pure nitrogen caused the disappearance of only 5% of hydrocarbon.

The yield of nitrogen-containing compounds (usually deep yellow) amounted to only a few mg. in each case. If we formulate the photo-nitroxides as A(NO) (A = hydrocarbon) the calculated nitrogen content of the primary product from 9 : 10-dimethyl-1 : 2-benzanthracene is 4.9%, and that from anthracene 6.7%. The nitrogen-content of most fractions was higher than the calculated values, indicating either the entry of more than one nitrogen-containing group into the molecule or a splitting of the ring structure with loss of carbon. From a preliminary experiment with anthracene, which was worked up immediately after irradiation, a substance was isolated with N, 5.72%. Its adsorption affinities, as judged by its position on the alumina column, were closer to those of anthracene than any of the other reaction products. As this substance was absent from the pooled irradiated solutions of later experiments, which were kept in the laboratory for some time, it must be labile. These properties are consistent with the assumption of its being identical with the primary photo-nitroxide. The somewhat low nitrogen content is probably due to contamination with unchanged anthracene, from which it is not easily separated by chromatography. The substance is of deep orange colour; it absorbs in the ultra-violet from 3500 to 4000 Å., with a peak at 3800 Å., and again at lower wave-lengths with a maximum at about 2500 Å. There is thus an overlapping of the absorption spectra of the hydrocarbon and the (presumed) photo-nitroxide, conditions which are very unfavourable for the preparation of the latter, in contrast to the sufficiently wide gap between the absorption spectra of the photo-peroxides and their parent hydrocarbons, which renders possible the stabilisation of the former.

EXPERIMENTAL.

Nitric oxide, prepared by addition of 40% sodium nitrite from a dropping funnel to a solution of 500 g. of hydrated ferrous sulphate in 1000 ml. of 10% sulphuric acid, was passed through two wash-bottles of concentrated sulphuric acid and two of 50% potassium hydroxide, and finally dried over phosphoric oxide. The components of the apparatus were joined by fusion or by ground-glass joints. After assembly, the apparatus was thoroughly rinsed out with pure nitrogen. At the end of an experiment the flask used for the generation of nitric oxide and the train of wash-bottles and drying tube were left filled with nitric oxide and shut off by a tap. The generating flask was provided with an outlet through which waste solution could be drawn off and replaced by fresh ferrous sulphate solution without admission of air. Before the vessel containing the irradiated solution was disconnected, the nitric oxide was quantitatively displaced by nitrogen.

Fluorescence measurements were carried out in the manner previously described (Weil-Malherbe, J., 1943, 303). Three readings were taken in each case: one in nitrogen, a second in nitric oxide, and a third again in nitrogen. Five minutes were allowed for saturation with the particular gas before the reading.

For the preparative experiments a rectangular, fused quartz cell of about 150 ml. capacity was used at a distance of about 40 cm. from a Hanovia mercury lamp. During irradiation the vessel was cooled by running water.

Examples of Preparative Irradiation Experiments.—(1) 9 : 10-Dimethyl-1 : 2-benzanthracene. 300 Mg. of hydrocarbon were dissolved in 100 ml. of hexane and irradiated in an atmosphere of nitric oxide for 4 hours. The slightly yellow solution was then twice extracted with 25 ml. of 2N-sodium hydroxide. The alkaline extract was immediately acidified, and repeatedly extracted with benzene. Both the hexane and the benzene solution were dried over calcium chloride and separately chromatographed on alumina columns. The hexane solution was poured on a column of 10 g. of alumina and treated with a series of solvents of increasing eluant power, *viz.*, hexane (1000 ml.), benzene—light petroleum (1 : 4; 100 ml.), benzene (10 + 20 ml.), ethyl acetate (10 + 15 ml.), methanol (100 ml.), and methanol with 10% acetic acid (100 ml.). A number of bands were formed, recognisable by their colour (yellow, orange, or brown) or fluorescence. Fractionation of the eluate was carried out according to the progress of the bands as observed in filtered ultra-violet light. The two benzene and the two ethyl acetate fractions were again separately chromatographed on smaller alumina columns and were thus resolved into 8 apparently homogeneous fractions. The fractions were then concentrated until crystallisation began or to dryness. The first two fractions consisted of unchanged hydrocarbon (235 mg.). From the second benzene fraction 1.3 mg. of orange needles (Found: N, 6.6%) were isolated. In the corresponding fraction of another experiment the nitrogen content was 6.50%. The first ethyl acetate fraction yielded 5 mg. of lemon-yellow crystals (Found: N, 5.3%). The second ethyl acetate fraction yielded 1 mg. of colourless, nitrogen-free crystals, m. p. 180° (uncorr.). From the methanol fraction 7.2 mg. of colourless, nitrogen-free crystals, insoluble in benzene, soluble in ethanol, were obtained; m. p. > 300°. The last fraction gave 5 mg. of difficultly soluble, amorphous or microcrystalline substance; this did not melt below 300° and did not contain nitrogen. All other fractions yielded only traces of oily smears.

The alkali-soluble fraction, after chromatographic adsorption and fractional elution, was resolved into a number of fractions which left only oily residues, except that the methanol fraction afforded 8 mg. of colourless crystals; these had m. p. $> 300^\circ$ and were nitrogen-free.

(2) *Anthracene*. 3.0 G. were dissolved in 1500 ml. of hexane and irradiated for 4 hours in batches of about 100 ml. The irradiated solutions were united and worked up together. During the irradiation a dark deposit formed on the walls of the vessel. It consisted of unchanged anthracene, which was washed out with hot hexane, and of more difficultly soluble material. It was treated first with hot benzene and then with hot ethanol. The united benzene washings were evaporated, the residue repeatedly extracted with hot hexane, and the hexane-insoluble part recrystallised from hot benzene; 65 mg. of colourless, nitrogen-free crystals (?dianthracene) were obtained, m. p. 267° (uncorr.) on rapid heating.

The main hexane solution was repeatedly extracted with 100-ml. portions of 2N-sodium hydroxide, the extract acidified, and extracted with 5 portions of 150 ml. of benzene. Hexane and benzene solutions were dried over calcium chloride. The hexane solution was chromatographed on a column of 100 g. of alumina, 2.5×21 cm. Elution proceeded on the lines detailed above, the following fractions being collected: hexane (2000 ml.), benzene-light petroleum (1:4; 1000 ml.), benzene (300 + 500 ml.), ether (1000 + 300 + 200 ml.), ethyl acetate (200 + 80 + 300 ml.), methanol (200 + 150 ml.). The column was then removed and divided into 3 parts, each being extracted several times with a total of about 400 ml. of hot methanol containing 10% acetic acid. With the exception of the hexane fraction, which contained only unchanged anthracene, and of the several methanol fractions, all intermediate fractions were evaporated, redissolved in either light petroleum or benzene, and again chromatographed on smaller columns (about 5 g. of alumina). Fractions from different columns which seemed identical were combined, and chromatography was repeated until the fractions were chromatographically homogeneous. On evaporation, 7 fractions yielded weighable amounts of crystalline products (Nos. 2-8, Table II), whereas several others either left only oily residues, usually dark, or too little crystalline material for investigation.

TABLE I.

Reversible quenching of fluorescence in nitric oxide.

Solute.	Concn., $\mu\text{g./5 ml.}$	Solvent.	Deflection (arbitrary units).			Quenching, %
			N_2 (1st reading).	NO.	N_2 (2nd reading).	
3 : 4-Benzpyrene	7.5	Hexane	353	23	345	93.5
	6	Decalin	352	82	344	76.7
	6	Ethanol	285	38	285	86.7
20-Methylcholanthrene	50	Hexane	243	24	170	90.0
	50	Decalin	404	90	350	77.7
	50	Ethanol	360	59	327	83.6
Anthracene	500	Hexane	315	92	310	70.8
	500	Decalin	355	213	355	40.0
	500	Ethanol	307	138	304	55.0
1 : 2-Benzanthracene	300	Hexane	270	16	266	94.1
	300	Ethanol	320	31	317	90.3
	150	Hexane	395	36	386	90.9
9 : 10-Dimethyl-1 : 2-benzanthracene	150	Ethanol	388	60	360	84.5
	500	Hexane	100	15	96	85.0
Naphthacene	Sat.	Hexane	62	18	56	71.0
Rubrene	300	Hexane	256	239	261	6.5
Ethylchlorophyllide	300	Ethanol	140	133	140	5.0
	50	Ethanol	30	21	30	30.0

TABLE II.

Fractions isolated after irradiation of anthracene in an atmosphere of nitric oxide.

No.	Yield (mg.).	M. p. (uncorr.).	Appearance.	Fluorescence of solution.	Reaction with conc. H_2SO_4 .	N, %.
1	9.0	139°	Orange needles	—	Rust-brown colour	5.72 †
2	2.0	261	Cream-coloured needles	Brilliant light blue	Yellow colour	0
3	36.5	279 *	Pale yellow needles	—	Yellow colour	0
4	1.5	276 (sint. 240°)	Cream-coloured needles	White	Yellow colour	?
5	6.1	244	Colourless plates (<i>ex</i> benzene)	Bright blue	No colour	8.35
6	7.0	108	Yellow needles (<i>ex</i> benzene)	Weak yellow	Ruby-red colour	14.9
7	10.3	163	Yellow prisms (<i>ex</i> ethanol)	—	Purple colour	10.55 †
8	6	227 (decomp.) (sint. 188°)	Colourless cubes (<i>ex</i> ethanol)	—	No colour	0
9	180	> 300	Colourless cubes (<i>ex</i> ethanol)	Weak grey-blue	No colour	0
10	28	> 300	Colourless plates (<i>ex</i> ethanol)	Blue	Yellow colour	0
11	67	> 300	Colourless, pale yellow crystals (octahedra, hexagons, stars like ice crystals, and various other forms (<i>ex isobutanol</i>))	Weak greyish	Effervescence	0
12	0.5	> 300	Yellow needles (<i>ex</i> ether)	Yellow	Red colour	?
13	68	> 300	Colourless needles or prisms in comb-like or fishbone-like arrangement (<i>ex</i> ethanol)	—	No colour	0

* M. p. of specimen of anthraquinone, 279° ; mixed m. p. 279° (uncorr.).

† Found: C, 42.6; H, 4.87. Calc. for $\text{C}_{14}\text{H}_{10}\text{O}_2\text{N}_2$: C, 43.1; H, 4.92; N, 10.8%. U.V. absorption: band in region of 2700 Å.

‡ U.V. absorption: maxima at 2500 and 3800 Å.

A total of 2.52 g. of anthracene was recovered unchanged. The bulk was contained in the hexane fraction, but some of the later fractions contained small amounts. It was always the most weakly adsorbed constituent.

No crystalline products were obtained from either of the two methanol eluates. A fairly large crop of crystals was isolated from the methanol-acetic acid eluate of the lowest third of the column (Table II, No. 9). The extracts of the middle and the top third of the column left amorphous residues which could be redissolved only with great difficulty. They were extracted with large volumes of hot *isobutanol*, from which by fractional crystallisation a number of fractions were obtained; apart from differences in their microscopic appearance, these had similar properties, notably an effervescence on addition of concentrated sulphuric acid. As they did not contain nitrogen, they were not further investigated and are combined in Table II as fraction No. 11.

Fractions 1—11 (Table II) were all obtained from the alkali-insoluble fraction; they are arranged in the order of increasing adsorption on the alumina column. Fraction No. 1, which immediately followed the anthracene on the column, was absent when the united solutions from the large-scale experiment were worked up. It was actually isolated in a preliminary experiment in which 700 mg. of anthracene, dissolved in 150 ml. of hexane, were irradiated for 4 hours; at the end of the experiment 610 mg. of anthracene were recovered unchanged.

Chromatography of the alkali-soluble fraction yielded two crystalline products: fraction No. 12 which crystallised out from an ether eluate, and fraction No. 13 obtained from a methanol-10% acetic acid eluate. A number of other eluates left only oily residues.

Irradiation of 9:10-Dimethyl-1:2-benzanthracene in an Atmosphere of Nitrogen.—200 Mg. of hydrocarbon, dissolved in 80 ml. of hexane, were irradiated in pure nitrogen for 5 hours, chromatographed, and unchanged hydrocarbon recovered (191 mg.). The residue of the ethyl acetate fraction of the eluate was 8.5 mg. of a dark, oily mass, partly soluble in light petroleum. On spontaneous evaporation, a trace of colourless crystals was deposited from the light petroleum solution.

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